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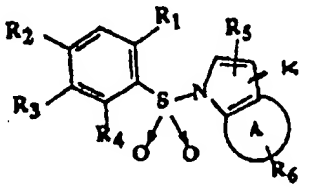
(54) Title: 1H-PYRROL-1-YL AND 1H-INDOL-1-YL ARYL SULPHONES, PROCESSES FOR THEIR PREPARATION AND USE FOR THE THERAPY OF HIV-1 INFECTIONS

(57) Abstract

This invention relates to new 1H-Pyrrol-1-yl and 1H-Indol-1-yl Aryl Sulphones of Formula (I) that may by useful in the medical therapy of retrovirus infections and in particular of HIV-1 infections. The compounds reported herein may be used alone or in combination with other antiretroviral compounds, preferably chosen among reverse transcriptase inhibitors such as, for example, nucleoside analogues, wherein: R^1 = NO_2, NH_2, halogen, $NHCH_2Z$(Z = H, alkyl, aryl, heteroaryl), NHCOW (W = H, alkyl, aryl, heteroaryl); R^2 = H, halogen; R^3 = R^4 = H, NO_2, NH_2, CH_3, halogen; R^5 = H, (2)-COX, (3)-COX, (X = OR, alkyl, aryl, CCl_3, N(alkyl$_2$); R = alkyl cycloalkyl, aryl arylmethyl; (2)-CONHY (Y = H, alkyl, aryl); R^6 = H, halogen, NO_2, NH_2, OCH_3; A = H, phenyl; K = H, CHO, $CH_2NC_5H_{11}$, $CH_2NC_4H_8NCH_3$.



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1H-PYRROL-1-YL AND 1H-INDOL-1-YL ARYL SULPHONES, PROCESSES FOR THEIR PREPARATION AND USE FOR THE THERAPY OF HIV-1 INFECTIONS.

TECHNICAL FIELD

This invention relates to new 1H-Pyrrol-1-yl and 1H-Indol-1-yl Aryl Sulphones that may be usefull in the medical therapy of retrovirus infections and in particular of HIV-1 infections.

BACKGROUND ART

Viral infections represent health problems whose solution depends on the development of vaccines and/or selective antiviral drugs, i.e. drugs that inhibit the multiplication of viruses without interfering with the growth of normal cells.

Among viral infections, the AIDS pandemic has rised widespread concern and, following the identification of human retroviruses as its causative agents, several targets susceptible of selective inhibition have been identified in the multiplication cycle of human immunodeficiency viruses (HIV). One of these targets is the reverse transcriptase (RT), a virus-coded enzyme that can be inhibited by two different groups of compounds.

The first group consists of nucleoside analogues such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (ddI), 2',3'-dideoxycytidine (ddC), 2',3'-didehydro-2',3'-dideoxythymidine (D4T) which, upon activation by cellular kinases, compete with natural substrates and efficiently inhibit the reverse transcription of both HIV-1 and HIV-2. Compounds of this group have been described by Mitsuya *et al.*, PNAS 82, 7096-7100, 1985; Yarchoan *et al.*, Science 245, 412-415, 1989; Mitsuya *et al.*, PNAS 83, 1911-1915, 1986; Lin. *et al.*, Biochem.Pharmacol. 36, 2713-2718, 1987.

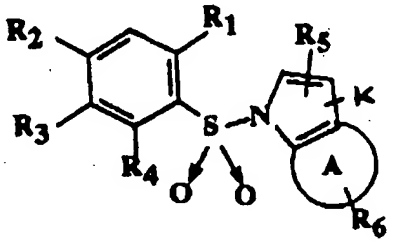
The second group comprises non-nucleoside RT inhibitors (NNRTI) such as 1-[(2-hydroxy-ethoxy) methyl]-6-phenylthio)thymine (HEPT), tetrahydroimidazo-[4,5,1-*jk*]-[1-4]-benzodiazepine-2(1*H*)-one and thione (TIBO), 6,11-dihydro-11-cyclopropyl-4-methyldipyrido-[2-3-b:2',3'-e]-[1,4]-diazepin-6-one (neviparine), bis-heteroaryl-piperazine (BHAP), which do not need activation by cellular enzymes, do not compete for the dNTP substrate site and specifically inhibit the multiplication of HIV-1 but neither of HIV-2 nor of other retro, RNA or DNA viruses. Compounds of this group have been described by Miyasaka *et al.*, J. Med. Chem. 32, 2507-2509, 1989;

Pauwels *et al.*, Nature 343, 470–474, 1990; Merluzzi *et al.*, Science, 250, 1411–1413, 1990; Romero *et al.*, PNAS, 88, 8806–8810, 1991.

The development of toxicity, which is a major problem with the nucleoside analogues, and the emergence of resistant mutants, which is of major concern with the NNRTI, are the most significant clinical limits of the above RT inhibitors. For this reason, the search for new antiviral compounds and the development of suitable therapeutic strategies aimed at reducing toxicity and preventing drug resistance is still a priority in the fight against AIDS.

SUMMARY

This invention relates to new compounds of general formula (I)



(I)

wherein:

R^1 = NO_2, NH_2, halogen, $NHCH_2Z$ (Z = H, alkyl, aryl, heteroaryl), NHCOW (W = H, alkyl, aryl, heteroaryl);

R^2 = H, halogen;

R^3 = R^4 = H, NO_2, NH_2, CH_3, halogen;

R^5 = H, (2)–COX, (3)–COX, (X = OR, alkyl, aryl, CCl_3, $N(alkyl_2)$); R = alkyl, cycloalkyl, aryl, arylmethyl; (2)–CONHY (Y= H, alkyl, aryl);

R^6 = H, halogen, NO_2, NH_2, OCH_3;

A = H, phenyl.

K = H, CHO, $CH_2NC_5H_{11}$, $CH_2NC_4H_8NCH_3$

The compounds of the invention may be usefull in the therapy of retrovirus infections, and in particular of HIV–1. They may be used alone or in combination with other

antiretroviral compounds. such as reverse transcriptase inhibitors and, in particular, nucleoside analogues.

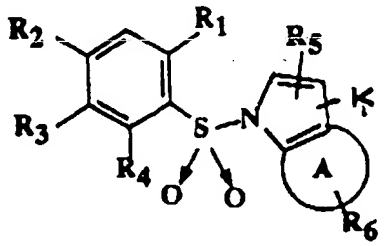
BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B represent the behaviour of p24 antigen by various types of treatment.

Figures 2A and 2B represent the behaviour of HIV-1 gag sequences by various types of treatment.

DETAILED DESCRIPTION OF THE INVENTION

The present invention allows to circumvent the prior art limits through the use of compounds of general formula (I)



(I)

wherein:

R^1 = NO_2, NH_2, halogen, $NHCH_2Z$ (Z = H, alkyl, aryl, heteroaryl), NHCOW (W = H, alkyl, aryl, heteroaryl);

R^2 = H, halogen;

R^3 = R^4 = H, NO_2, NH_2, CH_3, halogen;

R^5 = H, (2)-COX, (3)-COX, (X = OR, alkyl, aryl, CCl_3, $N(alkyl_2)$); R = alkyl, cycloalkyl, aryl, arylmethyl; (2)-CONHY (Y= H, alkyl, aryl);

R^6 = H, halogen, NO_2, NH_2, OCH_3;

A = H, phenyl.

K = H, CHO, $CH_2NC_5H_{11}$, $CH_2NC_4H_8NCH_3$

Compounds of formula (I) and their chemical and physical charcteristics are reported in tables 1 and 2, respectively.

Compounds of general formula (I) may be prepared by the processes described below.

Nitroaryl pyrrolyl sulfones 1-35 were synthesized by reaction of respective benzenesulfonyl chlorides with alkyl pyrrole-2-carboxylates and 2-acetylpyrrole in the presence of potassium *tert*-butoxide and 18-crown-6 (Scheme 1).

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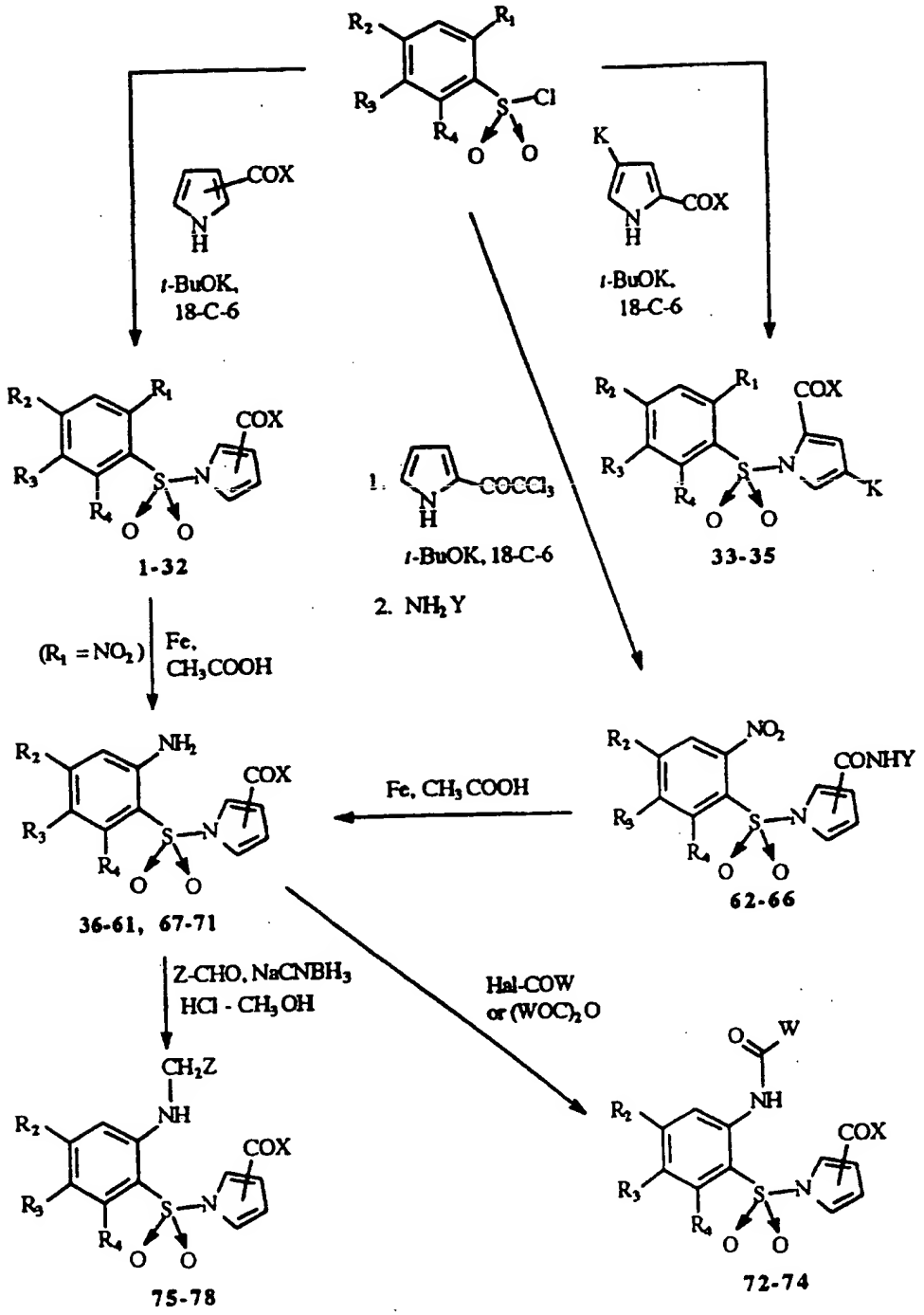
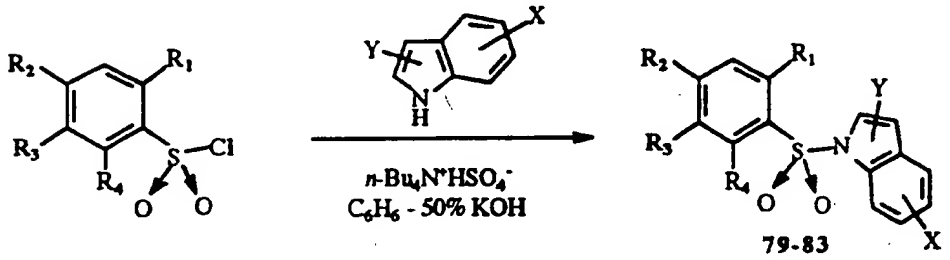
Scheme 1

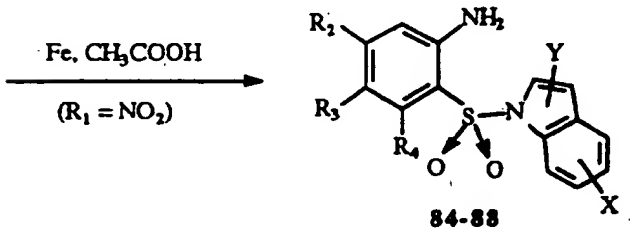


X = OR, alkyl, aryl, CCl_3, $CON(alkyl)_2$;
R = alkyl, *cyclo*alkyl, aryl, arylmethyl;
Y = H, alkyl, aryl;
Z = H, alkyl, aryl, heteroalkyl;
W = H, alkyl, aryl, heteroaryl;
K = CHO, CH_2OH.

Nitroaryl indolyl sulfones 79–83 were obtained by phase transfer–reaction of respective benzenesulfonyl chlorides with indole or ethyl indole–2–carboxylate in the presence of *n*–tetrabutylammonium hydrogen sulfate in benzene – aqueous 50% potassium hydroxide medium (Scheme 2).

Scheme 2





X = H, Cl, NO_2, OCH_3, NH_2;
Y = H, COOR, $CONH_2$, CONHR, COR;
R = H, alkyl, aryl, arylmethyl.

Displacement of trichloromethyl group of 1-(2-nitrobenzenesulfonyl)-2-trichloroacetyl-1H-pyrrole by amines afforded the corresponding amides **62-66.**

Iron powder reduction of nitro derivatives in glacial acetic acid by heating at 60°C for 2 h furnished the related anilines **36-61, 67-71** and **84-88.**

Reaction of ethyl 1-(2-amino-5-chlorobenzenesulfonyl)-1H-pyrrole-2-carboxylate with formaldehyde in the presence of sodium cyanoborohydride in methanol-hydrochloric acid afforded the required N-methyl derivative **76.** In a similar way were prepared the N-alkyl derivatives **75, 77** and **78.**

Amides **72-74** were obtained by refluxing 1-(2-amino-5-chlorobenzenesulfonyl)-1H-pyrrole-2-carboxylate with acyl chlorides in pyridine (73 and 74) or by treating with aceto formic anhydride (**72**).

The processes for the preparation of the compounds according to the present invention are illustrated by the following examples.

Example 1

Condensation of arylsulfonyl chlorides with 1*H*-pyrrole-2-carboxylic esters and 2-acetylpyrrole. Methyl 1-(2-Nitrobenzenesulfonyl)-1*H*-pyrrole-2-carboxylate.

A solution of 2-methoxycarbonyl-1*H*-pyrrole (12.50 g, 0.10 mol) in dry THF (210 mL) was added dropwise to a well-stirred mixture of potassium *tert*-butoxide (13.46 g, 0.10 mol) and 18-crown-6 (2.83 g, 0.01 mol) in the same solvent (210 mL). After 15 min the suspension was cooled on an ice-bath and then treated by dropping with a solution of 2-nitrobenzenesulfonyl chloride (22.16, 0.10 mol) in dry THF (210 mL). Stirring was continued at room temperature for 3.5 h, then the mixture was concentrated to a small volume and the residue was shaken between ethyl acetate and water. The organic layer was separated, washed with brine and dried. Removal of the solvent furnished the crude product which was purified by chromatography on alumina (chloroform). Yield 58%, mp 143°C (toluene/cyclohezane). ^{1}H-NMR (DMSO-d$_6$): δ 3.61 (s, 3H), 6.56 (t, 1H), 7.26 (m, 1H), 7.75-8.05 (m, 4H), 8.16 ppm (dd, 1H). IR: ν 1720 cm^{-1} (CO). Anal. $C_{12}H_{10}N_2O_6S$ (310.28) C, H, N, S.

Example 2

Condensation of benzenesulfonyl chlorides with indole and ethyl 2-indolecarboxylate. 1-(2-Nitrobenzenesulfonyl)-1*H*-indole.

50% KOH (20 mL) was dropped while stirring into a solution of indole (2.34 g, 0.02 mol) and *n*-tetrabutylammonium hydrogen sulfate (0.68 g, 0.002 mol) in benzene (40 mL). After 5 min a solution of 2-nitrobenzenesulfonyl chloride (4.43 g, 0.02 mol) in benzene (20 mL) was added dropwise. Reaction was stirred at room temperature for 1 h. Every 20 min was added 2-nitrobenzenesulfonyl chloride (2.21 g, 0.01 mol) in the same solvent (10 mL). The mixture was diluted with water and the organic layer separated, washed with brine and dried. Removal of the solvent gave the crude product which was purified by passing through an alumina column (chloroform). Yield 93%, mp 98-100°C (toluene/cyclohexane). ^{1}H-NMR ($CDCl_3$): δ 6.74 (d, 1H), 7.22-7.38 (m, 2H), 7.54-7.78 (m, 6 H), 7.85 ppm (m, 1H). Anal. $C_{14}H_{10}N_2O_4S$ (302.30) C, H, N, S.

Example 3

Reduction of nitro group into amino. 1-(2-Amino-4-chlorobenzenesulfonyl)-1*H*-pyrrole.

Iron powder (5.2 g) was added over a period of 15 min to a stirred solution of 1-(2-nitro-4-chlorobenzenesulfonyl)-1H-pyrrole (5.00 g, 0.017 mol) in glacial acetic acid (50 mL) while heating at 60°C, then the mixture was maintained at 60°C for 2 h. After evaporation of the solvent, the residue was shaken between ethyl acetate and water. Organic extracts were separated, washed with brine and dried. The residue was purified on alumina column (chloroform). Yield 83%, mp 167-168°C (toluene/ligroin). ^{1}H-NMR (DMSO-d_6): δ 6.31 (t, 2H), 6.58-6.65 (m, 3H), 6.88 (d, 1H), 7.38 (t, 2H), 7.65 ppm (d, 1H). IR: ν 3380, 3480 cm^{-1} (NH_2). Anal. $C_{10}H_9ClN_2O_2S$ (256.71) C, H, N, Cl, S.

Example 4

Alkylation of aminosters. 1-(2-Methylaminobenzenesulfonyl)-1*H*-pyrrole.

$NaBH_3CN$ (0.32 g, 0.005 mol) Was carefully added into a mixture of 1-(2-aminobenzenesulfonyl)-1H-pyrrole (1.00 g, 0.0045 mol), 37% aqueous formaldehyde (0.4 mL), 6N HCl / CH_3OH 1:1 (0.74 mL), methanol (18 mL), then reaction was stirred

at room temperature for 36 h. After concentration to a small volume the mixture was extracted with chloroform, washed with 5% $NaHCO_3$, then with brine and dried. Removal of the solvent furnished a residue which was purified on alumina column (dichloromethane/petroleum ether 1:1). Yield 45%, mp 134°C (ligroin). ^1H-NMR ($CDCl_3$): δ 2.87 (d, 3H), 6.15–6.35 (m, 3H), 6.67 (m, 2H), 7.15 (t, 2H), 7.40 (m, 1H), 7.72 ppm (dd, 1H). IR: ν 3440 cm^{-1} (NH). Anal. $C_{11}H_{12}N_2O_2S$ (236.28) C, H, N, S.

Example 5

Acylation of aminosters. Example. Ethyl 1-(2-Acetamido-5-chloro-benzenesulfonyl)-1*H*-pyrrole-2-carboxylate.

Acetyl chloride (1.17 g, 0.0148 mol) was dropped into an ice cooled solution of 1-(2-amino-5-chlorobenzenesulfonyl)-1H-pyrrole-2-carboxylate (1.00 g, 0.003 mol) in dry pyridine (10 mL), the reaction was refluxed overnight. After cooling mixture was poured on crushed ice, made acid with 12N HCl and shaken with ethyl acetate. Organic layer was separated, washed with brine and dried. After evaporation of the solvent, the crude product was purified on silica gel column (chloroform). Yield 84%, mp 119–121°C (cyclohexane). ^1H-NMR ($CDCl_3$): δ 1.26 (t, 3H), 2.23 (s, 3H), 4.18 (q, 2H), 6.36 (t, 1H), 7.09 (m, 1H), 7.43–7.55 (m, 2H), 7.71 (m, 1H), 8.42 (d, 1H), 9.47 ppm (br s, 1H). IR: ν 1650, 1720 (CO), 3250 cm^{-1} (NH). Anal. $C_{15}H_{15}ClN_2O_5S$ (370.80) C, H, N, Cl, S.

BIOLOGICAL ACTIVITY

In order to describe the usefullness of compounds of formula (I) for the treatment of infections caused by retroviruses, herein will be presented the results of experiments aimed at evaluating:

- potency, selectivity, spectrum and mode of anti-HIV activity;
- cytotoxicity for normal human cells;
- efficacy in suppressing the HIV-1 infection in long-term cultures.

Compounds were solubilized in DMSO at an initial concentration of 100 mM and then were serially diluted in RPMI 1640.

The following cell lines were used in cytotoxicity and antiviral assays: C8166 and MT-4, human $CD4^+$ T-cell lines purchased from the American Type Culture Collection (ATCC). Cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100

units/mL penicillin and 100 μg/mL streptomycin. The cultures were incubated at 37 °C in a humidified, 5% CO_2 atmosphere. The absence of mycoplasma contamination was checked periodically by the Hoechst staining method.

Human immunodeficiency viruses type-1 (HIV-1, III_B strain) and type 2 (HIV-2, CBL-20 and ROD strains) were obtained from the supernatant of persistently infected H9/III_B and CEM cells, respectively. Additional laboratory strains (MN, RF and 105/F, the latter an AZT-resistant strain) and a clinical isolate (CAMAS) were used. The HIV stock solutions were titrated in C8166 cells and kept at -80 °C until use.

Anti-HIV activity, spectrum and mode of action.

The activity of compounds against the HIV-1 multiplication in acutely infected cells was based on inhibition of the virus-induced cytopathogenicity (CPE) in MT-4 cells. Brielfly, 50 μL of culture medium additioned of 10% FCS and containing $1x10^4$ MT-4 cells were added to each well of flat bottomed microtitre trays containing 50 μL of medium without or with serial concentrations of the test compounds. 20 μL of a viral suspension were then added to give 100 $CCID_{50}$/well. After 4 days incubation at 37 °C, the number of viable MT-4 cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method (Pauwels R. *et al.*, J. Virol. Meth. 20, 309-321, 1988). Alternatively, cell-free culture supernatants were assayed by the HIV-1 p24 antigen enzyme-linked immunosorbent assay (ELISA, Abbott). Cytotoxicity of compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method. As shown in Table 3, representative compounds of the invention were found non-cytotoxic for MT-4 cells at concentrations higher than 100 μM (see CC_{50} column). Many of them were active against the HIV-1 multiplication at concentrations ranging from 1 to 20 μM (see EC_{50} column), whereas five compounds turned out highly potent showing EC_{50} in the submicromolar range.

In order to determine whether these compounds were targeted at the RT, they were tested against the HIV-1 recombinant reverse transcriptase (rRT). Assays were performed at 37° for 30 min. in a 50 μL reaction mixture containing 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 80 mM KCl, 6 mM $MgCl_2$, 0.1 mg/mL BSA, 10 mM [^{3}H]-dTTP (1 Ci/mmol), 0.05 OD_{260} units/mL of Poly(rA)-oligo$(dT)_{10}$ and $1.6x10^{-3}$ units of enzyme (a unit was defined as the amount of enzyme necessary to incorporate 1 nmol of [^{3}H]-dTMP into the

Poly(rA)–oligo(dT)$_{10}$ template in 1 min. at 37°). 40 μL aliquots were spotted on glass fiber filters (Whatman GF/A) and processed for determination of trichloroacetic acid–insoluble radioactivity. With the exception of **72**, **73** and **74** (Table 3), all compounds resulted inhibitory to the rRT at concentrations (see IC_{50} column) comparable to those active in cell culture–based assays. This confirmed that compounds of formula (I) with $R^1 = NH_2/NO_2$ interfere with the HIV–1 multiplication by inhibiting the RT activity.

Table 3. Cytotoxicity and anti–HIV–1 activity of compounds of general formula (I).

Compd	[a]CC_{50}	[b]EC_{50}	[c]IC_{50}± SD	[d]SI
1	>300	5.0	7.5	>60
2	>300	15	22	>20
36	>300	22	19.5	>14
37	>300	9.9	8.0	>30
41	>300	0.18	0.45± 0.09	>1666
42	>300	0.08	0.40± 0.05	>3750
43	110	0.22	0.40± 0.14	500
44	>300	0.14	0.27± 0.10	>2140
45	100	0.40	0.90± 0.12	250
72	>300	1	>20	>300
73	>300	1	>20	>300
74	>300	1	>20	>300
Nevirapine	>300	0.3	0.60	>1000
AZT	>20	0.01		>2000

[a]Compound dose (μM) required to reduce the viability of mock–infected MT–4 cells by 50%, as determined by the MTT method.

[b]Compound dose (μM) required to achieve 50% protection of MT–4 cells from the HIV–induced cytopathicity, as determined by the MTT method. Data represent mean values for three separate experiments. Variation among triplicate samples was less than 12%.

[c]Compound dose (μM) required to inhibit the HIV–1 rRT activity by 50% ± standard deviation.

[d]Selectivity index: CC_{50}/EC_{50} ratio.

The anti-HIV activity of compounds 41, 42 and 72 was evaluated also against additional HIV-1 laboratory strains (MN, RF), an highly AZT-resistant strain (105/F), a clinical isolate (CAMAS) and two HIV-2 strains (CBL 20, ROD). All the HIV-1 strains resulted sensitive (Table 4), whereas the HIV-2 strains were both unsusceptible to inhibition by compounds of formula (I).

Table 4. Activity of compounds 41, 42 and 72 against different HIV strains

	[a]EC_{50}					
Compd	HIV-1				[b]HIV-2	
	[c]NM	[c]RF	[c]105/F	[d]CAMAS	CBL 20	ROD
41	0.22	0.24	0.09	0.35	>100	>100
42	0.03	0.09	0.08	0.08	>100	>100
72	0.9	1.4	0.8	1.0	>100	>100
AZT	0.01	0.007	>1.5	0.007	0.01	0.02

[a]Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.

[b]Compound dose (μM) required to achieve 50% protection of C8166 cells from the HIV-2-induced cytopathicity.

[c]Compound dose (μM) required to reduce the number of syncytia by 50%. Untreated cultures contained 80 syncytia/field.

[d]Compound dose (μM) required to reduce p24 levels by 50%.

The activity of compounds 41, 42 and 72 was evaluated in cultures infected at different multiplicities by measuring p24 antigen levels. Nevirapine, AZT ddI and ddC were used as reference drugs. Interestingly, the compounds of the invention were inhibitory to HIV-1 also in cells acutely infected at high multiplicities of infection (m.o.i.). In this respect they behaved similarly to nevirapine and AZT, but differently from ddI and ddC. In fact, at m.o.i. $\geq$ 1.0 the later were unable to prevent the viral breakthrough even when used at high concentrations.

Cytotoxicity for normal human cells.

In order to get more insights into the cytotoxic potential of compounds of formula (I), 41, 42 and 72 were tested in vitro against peripheral blood lymphocytes (PBL) from HIV-

negative donors and bone marrow granulocyte/monocyte (CFU-GM) precursors from healthy individuals. AZT was used as reference drug. PBL and CFU-GM were obtained by separation on Fycoll-Hypaque gradients. After extensive washings, cells were resuspended ($1x10^6$ cells/mL) in RPMI-1640 with 10% FCS and incubated overnight to allow adhesion of the macrophages to the plastic.

Table 5. Activity of compounds 41, 42 and 72 in cultures infected with HIV-1 at different multiplicities of infection.

Compd	$^*EC_{50}$		
	m.o.i. 0.01	m.o.i. 0.1	m.o.i. 1.0
41	0.18	0.4	1.2
42	0.08	0.3	0.9
72	0.9	1.9	4.7
Nevirapine	0.1	0.2	0.7
AZT	0.008	0.02	0.09
ddI	5.2	15	>20
ddC	1.2	3.7	>10

*Compound dose (μM) required to reduce the level of p24 antigen by 50% at day 4 post infection. Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.

Cytotoxicity of compounds for activated PBL was evaluated by resuspending non-adherent cells at $1x10^6$ cells/mL in growth medium and stimulating with PHA (2.5 μg/mL) for 24 hrs before dilution to $1x10^5$ cells/mL in medium containing PHA (2.5 μg/mL), IL-2 (50 U/mL) and various concentrations of the test compounds. Viable cell numbers were determined six days later. Under these conditions, untreated PBL were able to undergo exponential growth for up to four cell cycles, as determined by viable cell counts. For cytotoxicity evaluations in resting PBL, non-adherent cells were resuspended at high density ($1x10^6$ cells/mL) and were treated for 3 days with the test compounds. Then, the cells were extensively washed to remove the inhibitors and were stimulated with PHA for 24 hrs before being diluted to $1x10^5$ cells/mL in medium containing PHA and IL-2. Cell viability was determined after incubation at 37°C for six days. CFU/GM ($2x10^5$/mL) were resuspended in Iscove medium containing 0.3% agar

and growth factors from the supernatant of 5637 cells. After ten days at 37°C, colonies (of about 40 cells) were scored under the light microscope.

Table 6. Cytotoxicity of compound **41, 42** and **72** for human cells.

Compd	[a]CC_{50}		
	[b]PBL_{PHA}	[c]$PBL_{resting}$	[d]CFU-GM
41	>100	>100	>100
42	>100	>100	>100
72	>100	>100	>100
AZT	[e]23	>100	[e]2.4

[a]Compound dose (μM) required to reduce cell growth (PBL) or colony formation (CFU-GM) by 50%.

[b]PHA-stimulated were resuspended in IL_2-containing medium in the presence of the drugs.

[c]PBL were treated with the test drugs for 3 days and then were stimulated with PHA and allowed to grow in drug-free medium.

[d]CFU-GM, colony forming units of bone marrow hematopoietic progenitors of granulocytes / macrophages.

[e] Data represent mean values for two separate experiments. Variation among duplicate samples was less than 14%.

As shown in Table 6, **41**, **42** and **72** proved non-cytotoxic (CC_{50} > 100 μM) for both PHA-stimulated and resting PBL, whereas AZT resulted cytotoxic for activated PBL. Moreover, when the cytotoxicity of each compound for myeloid precursor cells was evaluated, compounds of formula (I) resulted non-cytotoxic, whereas AZT showed a CC_{50} of 2.4 μM.

Efficacy in suppressing the HIV-1 infection.

The long-term anti-HIV-1 activity of compound **42** and AZT, alone and in combination, was evaluated in cultures of MT-4 cells and was based on both evaluation of p24 antigen levels and protection from the HIV-1-induced CPE, as monitored by the Elisa test and the MTT method, respectively. Briefly, $1x10^6$ MT-4 cells were infected with $1x10^6$ $CCID_{50}$ (1 $CCID_{50}$ = 25 -250 infectious virions) at 20 °C for 1 hr, washed three times, resuspended at $2x10^5$ cells/mL as such (m.o.i. ≥ 1) or after dilution 1:10 (m.o.i. =

0.1) or 1:100 (m.o.i. = 0.01) with medium containing $2x10^5$ uninfected cells/mL. $1x10^4$ cells/well were seeded in 96 multiwell plates and grown at 37°C in a humidified 5% CO_2 atmosphere in the absence or presence of the drugs, alone or in combination. 4 days later, the whole sample (0.1 mL) was resuspended in 0.9 mL of fresh medium containing the given drug concentrations and seeded in 24 multiwell plates. After 4 more days, the whole culture (1.0 mL) was resuspended in 9 mL of fresh medium (containing the given drug concentrations) in a 25-cm^2 flask. Starting at day 12, only one-tenth (in 25-cm^2 flasks) or one hundreth (in 24 multiwell plates) of each culture was further transplanted.

It is worth noting that the above procedure, while settling conditions suitable for continuous exponential growth of the cultures, on the other hand allowed to keep all the cells which were originally infected (or the virus produced by them) up to day 12 post infection (p.i.).

When used alone at 10 μM, 42 delayed up to day 12 the rise of p24 antigen (Fig. 1A) and the development of the virus-induced CPE (Fig. 1B). Under the same conditions, AZT 2.5 μM reduced to very low (although still detectable) levels the p24 antigen, prevented the virus-induced CPE and allowed exponential growth of the culture up to day 32 post infection (p.i.). However, when AZT was removed, the HIV-1 multiplication resumed within few days, as evidenced by the rise of p24 levels, production of infectious virus ($1.2x10^6$ $CCID_{50}$/mL) and cell death. In the experiment of Fig. 1 removal of AZT was carried out 20 days p.i., but analogous results were obtained when AZT was removed as early as 4 days p.i. and as late as 28 days p.i..

On the contrary, when the cells were treated with compound 42, 10 μM, in combination with AZT, 2.5 μM (Fig.1), total lack of both p24 antigen and HIV-1-induced CPE was observed for as long as 32 days. Interestingly, when HIV-1-infected cultures that had been treated with the above combination for 4 days were further transplanted in drug-free medium, no signs of resumption of viral multiplication became evident up to day 32 p.i..

PCR analyses of the experiment in Fig. 1 were in agreement with the above observations. The HIV-1 DNA was checked (as described by Bagnarelli *et al.*, J. Med. Virol. 34, 89-95. 1991; Menzo *et al.*, J. Clinical Microbiol. 30, 1752-1757, 1992) by using the set of primers SK38 and SK39, which amplify an internal, highly conserved fragment (115 bp) of the *gag* gene. The reaction mixture was subjected to 35 cycles of denaturation at 93°C

for 15 seconds, annealing at 60 °C for 15 seconds, extension at 72 °C for 30 seconds. The extension step of the last cycle was 10 minutes longer to ensure full completion of the newly synthesized strains. Amplified products were analyzed by electrophoresis on low melting point agarose gel and visualized by ethidium bromide staining.

As shown in Fig.2A, the treatment with AZT 2.5 μM was unable to prevent the synthesis of HIV-1 gag sequences, that could be evidenced from day 4 through day 32, no matter whether p24 antigen was barely detectable and infectious virus was absent. By contrast, the cultures treated with the 42-AZT combination remained free of gag DNA sequences starting from day 8 on. The same was true for the samples from which the drugs were removed by day 4 p.i. (Fig.2B).

Due to their characteristics, the compounds according to the present invention find a useful use in the therapy of retrovirus infections, used both alone or in combination with other antiretroviral compounds.

Therefore the present invention refers also to pharmaceutical compositions containing as active substance a compound having formula (I) as well as to a therapeutic method for treating the retrovirus infections.

Said pharmaceutical compositions are characterized by containing as active substance a pharmacologically effective amount of a substance of formula (I) in mixture with pharmacologically acceptable diluents and excipients.

Said therapeutic method consists in administering by oral route a dose of 10 mg per day and per Kg. of body weight of a compound of formula (I).

Table 1 - Chemical Structure of Derivatives 1-88

Compd	R	R^1	R^2	R^3	R^4	R^5	K	X	Y	Z	A
1	CH_3	NO_2	H	H	H	(2)COX	-	OR	-	-	H
2	C_2H_5	NO_2	H	H	H	(2)COX	-	OR	-	-	H
3	C_2H_5	Cl	H	H	H	(2)COX	-	OR	-	-	H
4	C_2H_5	F	H	H	H	(2)COX	-	OR	-	-	H
5	-	NO_2	H	H	H	(2)COX	-	CH_3	-	-	H
6	CH_3	NO_2	Cl	H	H	(2)COX	-	OR	-	-	H
7	C_2H_5	NO_2	Cl	H	H	(2)COX	-	OR	-	-	H
8	C_2H_5	NO_2	Cl	Cl	H	(2)COX	-	OR	-	-	H
9	CH_3	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
10	C_2H_5	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
11	n-C_3H_7	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
12	i-C_3H_7	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
13	CH_2ClCH_2	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
14	$CH_2C_6H_5$	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
15	$(CH_2)_2NEt_2$	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
16	C_2H_5	NO_2	H	CH_3	H	(2)COX	-	OR	-	-	H
17	CH_3	Cl	H	NO_2	H	(2)COX	-	OR	-	-	H
18	C_2H_5	Cl	H	NO_2	H	(2)COX	-	OR	-	-	H
19	n-C_4H_9	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
20	i-C_4H_9	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
21	s-C_4H_9	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
22	C_2H_5	NO_2	Cl	H	Cl	(2)COX	-	OR	-	-	H
23	C_6H_5	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
24	*cyclo*-C_5H_9	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
25	*cyclo*-C_6H_{11}	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
26	CH_2CCH	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
27	*cyclo*-$C_3H_5CH_2$	NO_2	H	Cl	H	(2)COX	-	OR	-	-	H
28	C_2H_5	NO_2	H	H	Cl	(2)COX	-	OR	-	-	H
29	C_2H_5	NO_2	H	Cl	H	(3)COX	-	OR	-	-	H

Table 1 – Chemical Structure of Derivatives 1–88

Compd	R	R^1	R^2	R^3	R^4	R^5	K	X	Y	Z	A
30	–	NO_2	H	Cl	H	(2)COX	–	CH_3	–	–	H
31	–	NO_2	H	Cl	H	(3)COX	–	CH_3	–	–	H
32	–	NO_2	H	Cl	H	(3)COX	–	C_6H_5	–	–	H
33	C_2H_5	NO_2	H	Cl	H	(2)COX	CHO	OR	–	–	H
34	C_2H_5	NO_2	H	Cl	H	(2)COX	$CH_2NC_5H_{11}$	OR	–	–	H
35	C_2H_5	NO_2	H	Cl	H	(2)COX	$CH_2NC_4H_8NCH_3$	OR	–	–	H
36	CH_3	NH_2	H	H	H	(2)COX	–	OR	–	–	H
37	C_2H_5	NH_2	H	H	H	(2)COX	–	OR	–	–	H
38	CH_3	NH_2	Cl	H	H	(2)COX	–	OR	–	–	H
39	C_2H_5	NH_2	Cl	H	H	(2)COX	–	OR	–	–	H
40	C_2H_5	NH_2	Cl	Cl	H	(2)COX	–	OR	–	–	H
41	CH_3	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
42	C_2H_5	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
43	n-C_3H_7	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
44	i-C_3H_7	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
45	CH_2CHCH_2	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
46	$CH_2C_6H_5$	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
47	$(CH_2)_2NEt_2$	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
48	C_2H_5	NH_2	H	CH_3	H	(2)COX	–	OR	–	–	H
49	CH_3	Cl	H	NH_2	H	(2)COX	–	OR	–	–	H
50	C_2H_5	Cl	H	NH_2	H	(2)COX	–	OR	–	–	H
51	n-C_4H_9	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
52	i-C_4H_9	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
53	s-C_4H_9	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
54	C_2H_5	NH_2	Cl	H	Cl	(2)COX	–	OR	–	–	H
55	C_6H_5	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
56	*cyclo*-C_5H_9	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
57	*cyclo*-C_6H_{11}	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
58	CH_2CCH	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H

Table 1 – Chemical Structure of Derivatives 1–88

Compd	R	R^1	R^2	R^3	R^4	R^5	K	X	Y	Z	A
59	*cyclo*-$C_3H_5CH_2$	NH_2	H	Cl	H	(2)COX	–	OR	–	–	H
60	C_2H_5	NH_2	H	H	Cl	(2)COX	–	OR	–	–	H
61	C_2H_5	NH_2	H	Cl	H	(3)COX	–	OR	–	–	H
62	–	–	H	H	H		–	–	H	–	H
63	–	–	H	Cl	H		–	–	H	–	H
64	–	–	H	Cl	H		–	–	C_2H_5	–	H
65	–	–	H	Cl	H		–	–	$(C_2H_5)_2$	–	H
66	–	–	H	Cl	H		–	–	$(CH_3)CH_2Ph$	–	H
67	–	NH_2	H	H	H	(2)COX	–	NH_2	–	–	H
68	–	NH_2	H	Cl	H	(2)COX	–	NH_2	–	–	H
69	–	NH_2	H	Cl	H	(2)COX	–	NHC_2H_5	–	–	H
70	–	NH_2	H	Cl	H	(2)COX	–	$N(C_2H_5)_2$	–	–	H
71	–	NH_2	H	Cl	H	(2)COX	–	$(CH_3)CH_2Ph$	–	–	H
72	C_2H_5	NHCHO	H	Cl	H	(2)COX	–	OR	–	–	H
73	C_2H_5	$NHCOCH_3$	H	Cl	H	(2)COX	–	OR	–	–	H
74	C_2H_5	$NHCOC_2H_5$	H	Cl	H	(2)COX	–	OR	–	–	H
75	–	–	H	H	H	(2)COX	–	OR	–	H	H
76	C_2H_5	–	H	Cl	H	(2)COX	–	OR	–	H	H
77	C_2H_5	–	H	Cl	H	(2)COX	–	OR	–	CH_3	H
78	C_2H_5	–	H	Cl	H	(2)COX	–	OR	–	n-C_3H_7	H
79	–	NO_2	H	H	H		–	H	H	–	Ph
80	–	NO_2	Cl	H	H		–	H	H	–	Ph
81	–	NO_2	H	Cl	H		–	H	H	–	Ph
82	–	NO_2	H	Cl	H		–	H	(2)-$COOC_2H_5$	–	Ph
83	–	Cl	H	NO_2	H		–	H	H	–	Ph
84	–	NH_2	H	H	H		–	H	H	–	Ph
85	–	NH_2	Cl	H	H		–	H	H	–	Ph
86	–	NH_2	H	Cl	H		–	H	H	–	Ph
87	–	NH_2	H	Cl	H		–	H	(2)-$COOC_2H_5$	–	Ph
88	–	Cl	H	NH_2	H		–	H	H	–	Ph

Table 2. Chemical and Physical Data of Derivatives 1-8C (page 1).

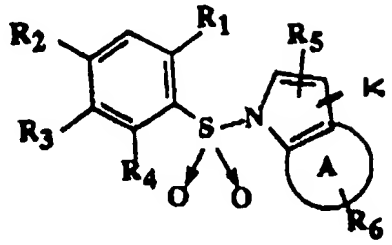
Compd	Mol. Weight	Cryst. Solvent	M. P. °C
1	310.28	toluene/cyclohexane	143
2	324.31	benzene/cyclohexane	120-122
3	313.75	ligroin	78-80
4	297.30	ligroin	61-62
5	294.28	toluene/cyclohexane	148-149
6	344.72	toluene/cyclohexane	149-150
7	358.75	toluene/cyclohexane	124-125
8	393.20	cyclohexane	115
9	344.72	toluene/cyclohexane	128-129
10	358.75	toluene/cyclohexane	121-122
11	372.78	cyclohexane	98-99
12	372.78	cyclohexane	132-133
13	370.76	cyclohexane	123-124
14	420.82	toluene/cyclohexane	149-147
15	429.87	ligroin	65-66
16	338.33	toluene/cyclohexane	99
17	344.72	toluene/cyclohexane	159-161
18	358.75	cyclohexane	120-121
19	386.80	cyclohexane	68-69
20	386.80	cyclohexane	98-99
21	386.80	toluene/cyclohexane	119-120
22	393.19	toluene/cyclohexane	135-136
23	406.80	toluene/cyclohexane	170-171
24	398.82	cyclohexane	98
25	412.84	cyclohexane	124-125
26	368.75	toluene/cyclohexane	156-157
27	384.79	cyclohexane	104-105
28	358.75	toluene/cyclohexane	151-153
29	358.75	-	oil
30	328.72	toluene/cyclohexane	185
31	328.72	toluene/cyclohexane	131-132
32	390.77	toluene/cyclohexane	158-159
33	386.76	toluene/cyclohexane	191-192
34	455.91	ligroin	117-119
35	470.92	toluene/cyclohexane	116-118
36	280.29	toluene/cyclohexane	119-120
37	294.32	aqueous ethanol	95-97
38	314.74	toluene/cyclohexane	113-114
39	328.77	ligroin	109
40	363.21	toluene/cyclohexane	132-133
41	314.74	toluene/ligroin	156-159
42	328.44	toluene/cyclohexane	143-145
43	342.80	cyclohexane	65-66
44	342.80	toluene/cyclohexane	127
45	340.78	cyclohexane	77
46	390.84	toluene/cyclohexane	92-93
47	399.89	ligroin	80-81
48	308.35	toluene/cyclohexane	117
49	314.74	toluene/cyclohexane	157
50	328.77	toluene/ligroin	110-111
51	356.82	cyclohexane	102
52	356.82	cyclohexane	90-92
53	356.82	cyclohexane	118
54	363.21	toluene/cyclohexane	147-148
55	376.81	toluene/cyclohexane	125-126
56	368.83	toluene/cyclohexane	122-123
57	382.86	toluene/cyclohexane	156-157
58	338.76	toluene/cyclohexane	103-105
59	354.80	cyclohexane	92-93
60	328.77	cyclohexane	103-104
61	328.77	toluene/cyclohexane	120
62	295.27	ethanol	216-217
63	329.71	ethanol	195-196

Table 2. Chemical and Physical Data of Derivatives 1-82 (page 2).

64	357.77	toluene/cyclohexane	170-172
65	358.82	cyclohexane	113-114
66	433.86	toluene/cyclohexane	139-141
67	265.29	ethanol	133-134
68	299.73	ethanol	173-175
69	327.62	ethanol	158-160
70	355.84	toluene/cyclohexane	142-145
71	403.88	toluene/cyclohexane	134-136
72	356.78	toluene/cyclohexane	109-111
73	370.80	cyclohexane	119-121
74	442.87	cyclohexane	89-90
75	236.28	ligroin	134
76	342.79	cyclohexane	119-120
77	356.82	ligroin	128-129
78	384.87	ligroin	98-99
79	302.30	toluene/cyclohexane	98-100
80	336.74	toluene/cyclohexane	134
81	336.74	toluene/cyclohexane	110-111
82	408.81	toluene/cyclohexane	157-158
83	336.74	toluene/cyclohexane	134-134
84	272.32	toluene/cyclohexane	115-116
85	306.76	toluene/cyclohexane	143-145
86	306.76	toluene/cyclohexane	106-107
87	378.83	cyclohexane	77-78
88	306.76	toluene/cyclohexane	117-118

CLAIMS

1. Compounds having general formula (I)



(I)

wherein:

$R^1 = NO_2$, NH_2, halogen, $NHCH_2Z$ (Z = H, alkyl, aryl, heteroaryl), NHCOW (W = H, alkyl, aryl, heteroaryl);

R^2 = H, halogen;

$R^3 = R^4$ = H, NO_2, NH_2, CH_3, halogen;

R^5 = H, (2)COX, (3)–COX, (X = OR, alkyl, aryl, CCl_3, N(alkyl$_2$)); R = alkyl, cycloalkyl, aryl, arylmethyl; (2)–CONHY (Y= H, alkyl, aryl);

R^6 = H, halogen, NO_2, NH_2, OCH_3;

A = H, phenyl.

K = H, CHO, $CH_2NC_5H_{11}$, $CH_2NC_4H_8NCH_3$

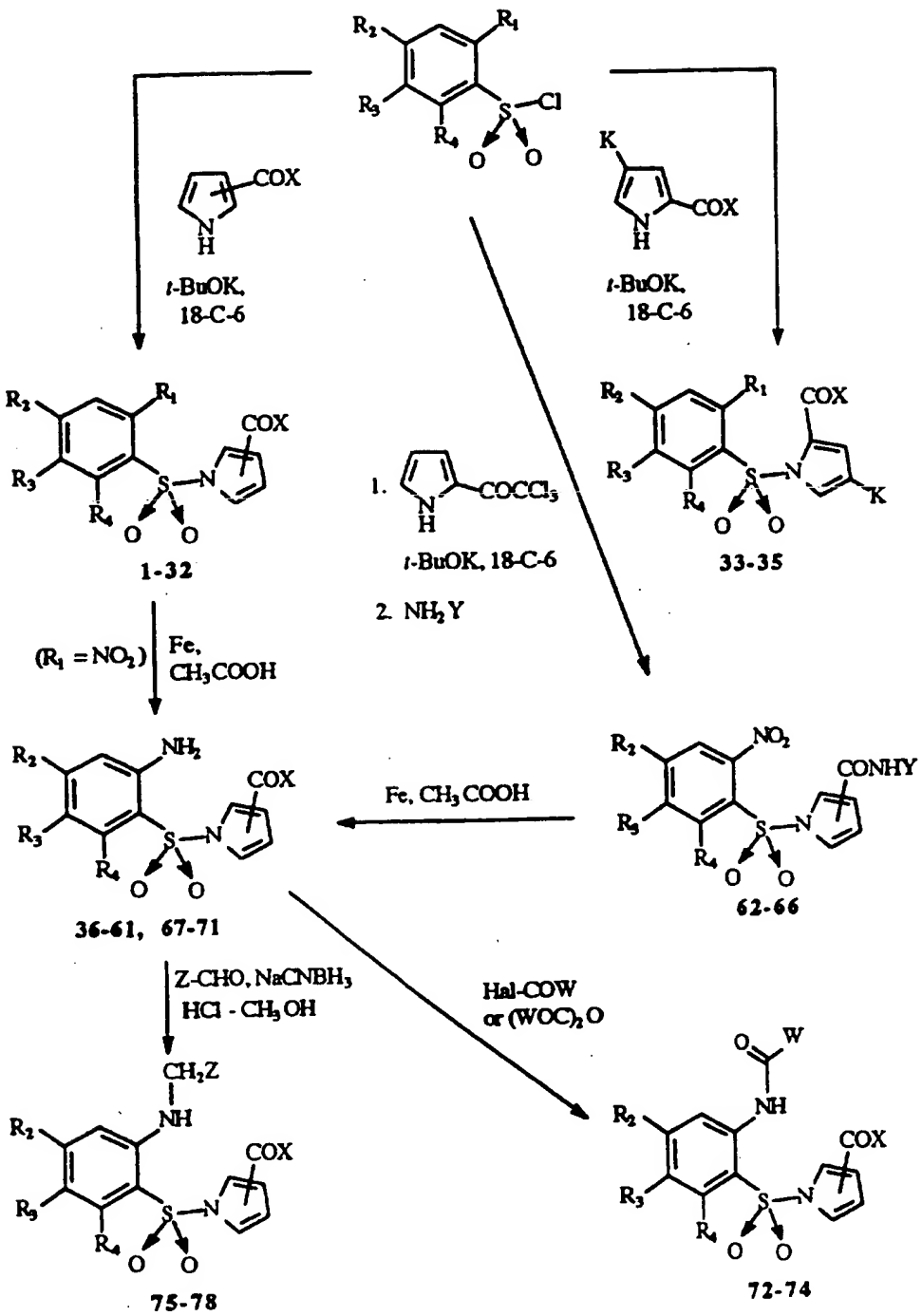
2. Compounds according to claim 1, wherein: $R^1 = NH_2$; R^2 = H; R^3 = Cl; R^4 = H; R^5 = (2)COX with X = OCH_3 and A = H

3. Compounds according to claim 1, wherein: $R^1 = NH_2$; R^2 = H; R^3 = Cl; R^4 = H; R^5 = (2)COX with X = OC_2H_5 and A = H

4. Compounds according to claim 1, wherein: R^1 = NHCHO; R^2 = H; R^3 = Cl; R^4 = H; R^5 = (2)COX with X = OC_2H_5 and A = H

5. Process for the preparation of compounds having general formula (I) according to claim 1, characterized by the steps of the following scheme (1).

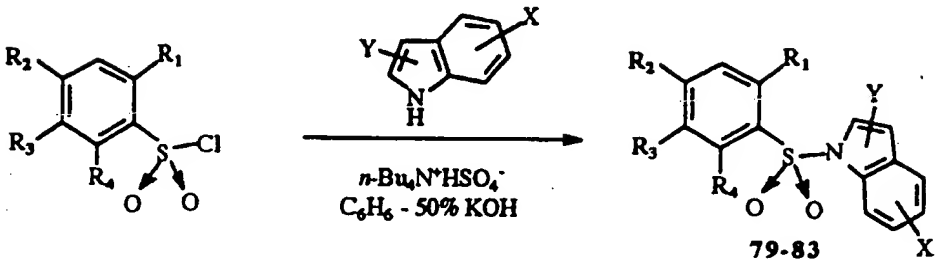
Scheme 1

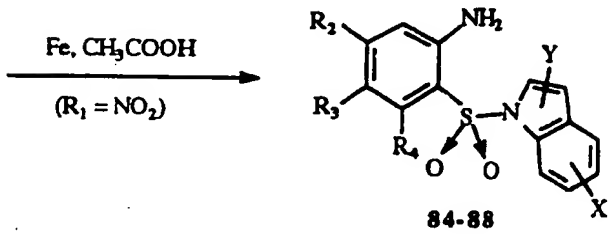


X = OR, alkyl, aryl, CCl_3, $CON(alkyl)_2$;
R = alkyl, *cyclo*alkyl, aryl, arylmethyl;
Y = H, alkyl, aryl;
Z = H, alkyl, aryl, heteroalkyl;
W = H, alkyl, aryl, heteroaryl;
K = CHO, CH_2OH.

6. Process for the preparation of compounds having general formula (I), characterized by the steps of the following steps (2).

Scheme 2





X = H, Cl, NO_2, OCH_3, NH_2;
Y = H, COOR, $CONH_2$, CONHR, COR;
R = H, alkyl, aryl, arylmethyl.

7. Pharmaceutical composition usefull in the medical therapy of retrovirus infections characterized by containing as active substance a pharmacologically effective amount of a substance according to claim 1 in mixture with pharmacologically acceptable diluents and excipients.

8. Therapeutic method for the medical treatment of retrovirus infections consisting of administering by oral route a dose of 10 mg per day and per Kg. of body weight of a compound according to claim 1.

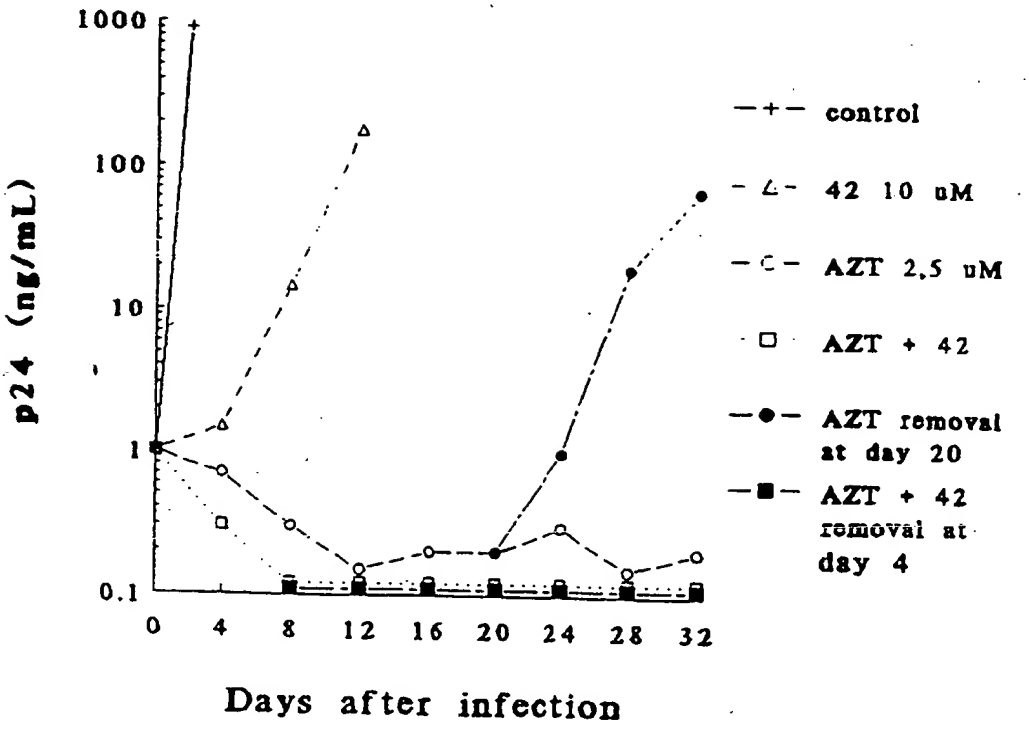

1000
100
10
1
0.1
p24 (ng/mL)
0 4 8 12 16 20 24 28 32
Days after infection
control
42 10 uM
AZT 2,5 uM
AZT + 42
AZT removal at day 20
AZT + 42 removal at day 4

FIG. 1A

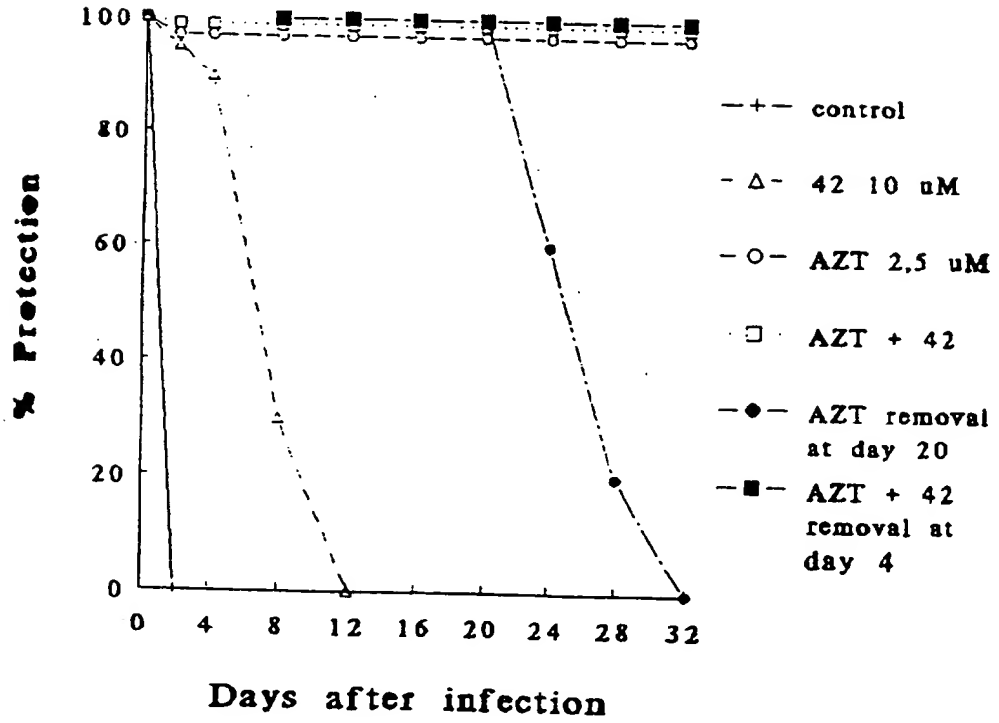

100
80
60
40
20
0
% Protection
0 4 8 12 16 20 24 28 32
Days after infection
control
42 10 uM
AZT 2,5 uM
AZT + 42
AZT removal at day 20
AZT + 42 removal at day 4

FIG. 1B

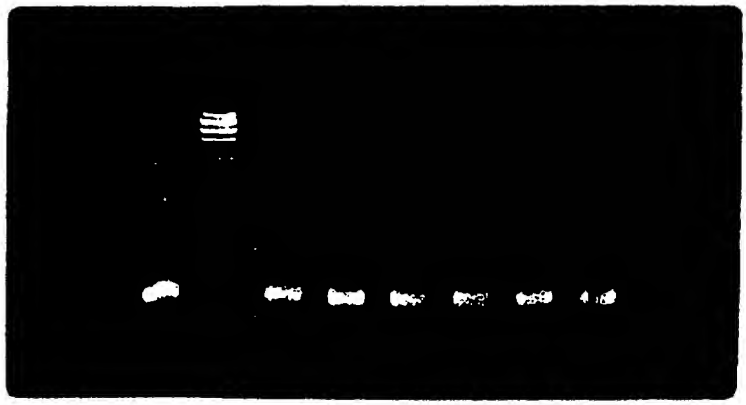

4 8 12 16 20 32
DAYS AFTER INFECTION

FIG. 2A


4 8 12 16 20
DAYS AFTER INFECTION
4 8 12 16
DAYS AFTER DRUG REMOVAL

FIG. 2B

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/EP 96/01642

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D207/48 C07D209/42 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J. MED. CHEM. (1996), 39(2), 522-30 CODEN: JMCMAR;ISSN: 0022-2623, 19 January 1996, XP000576074 ARTICO, MARINO ET AL: "2-Sulfonyl-4-chloroanilino moiety: A potent pharmacophore for the anti-human immunodeficiency virus Type 1 activity of pyrrolyl aryl sulfones." see the whole document ---	1-8
X	ARCH. PHARM. (WEINHEIM, GER.) (1995), 328(3), 223-9 CODEN: ARPMAS;ISSN: 0365-6233, March 1995, XP000576697 ARTICO, MARINO ET AL: "Synthesis of pyrryl aryl sulfones targeted at the HIV-1 reverse transcriptase" see the whole document ---	1,5-8
	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 1996	07.08.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Kissler, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. nal Application No
PCT/EP 96/01642

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. HETEROCYCL. CHEM. (1994), 31(4), 1033-6 CODEN: JHTCAD;ISSN: 0022-152X, 1994, XP002009323 SILVESTRI, ROMANO ET AL: "Heterocycles with a benzothiadiazepine moiety. 3. Synthesis of imidazo[5,1-d]pyrrolo[1,2-b][1,2,5]benzothiadiazepine 9,9-dioxide" scheme 1,2	1,5,6
X	SYNTH. COMMUN. (1992), 22(10), 1433-9 CODEN: SYNCAV;ISSN: 0039-7911, 1992, XP000576666 ARTICO, MARINO ET AL: "Heterocycles with a benzothiadiazepine moiety. 1. Synthesis of pyrrolo[1,2-b]-s-triazolo[3,4-d][1,2,5]benzothiadiazepine 5,5-dioxide" scheme 1	1,5,6
X	FARMACO, ED. SCI. (1974), 29(8), 589-97 CODEN: FRPSAX, 1974, XP000576049 CHIMENTI, F. ET AL: "Compounds with antiblastic activity. LVII. Anthramycin and related compounds. VI. Synthesis of pyrrolo[1,2-b][1,2,5]benzothiadiazepine derivatives" chart 2,3; compound (X); chart 4	1,5,6
X	SYN. COMMUN. (1973), 3(4), 303-4 CODEN: SYNCAV, 1973, XP000576672 WASLEY, JAN W. F. ET AL: "Synthesis of 1-arylsulfonylpyrroles" see the whole document	1,5,6

2

INTERNATIONAL SEARCH REPORT

Inte. onal application No.
PCT/EP 96/01642

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 8 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

(19) BUNDESREPUBLIK DEUTSCHLAND

PATENTSCHRIFT

(12) Ausschließungspatent

(11) DD 298 913 A5

Erteilt gemäß § 17 Absatz 1
Patentgesetz der DDR
vom 27.10.1983
in Übereinstimmung mit den entsprechenden
Festlegungen im Einigungsvertrag

5(51) C 07 D 207/34
A 61 K 31/40

DEUTSCHES PATENTAMT

In der vom Anmelder eingereichten Fassung veröffentlicht

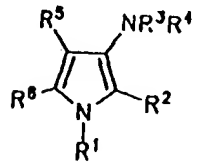
(21)	DD C 07 D / 338 218 2	(22)	17.11.89	(44)	19.03.92

(71) siehe (73)
(72) Liebscher, Jürgen, Doz. Dr. sc. nat. Dipl.-Gwl., DE; Knoll, Alexander, Dr. rer. nat. Dipl.-Chem., SU; Uschmajew, Alexej, Dr. rer. nat. Dipl.-Chem., SU; Rolfs, Andreas, Dipl.-Lebensm.-Chem., DE; Lohmann, Dieter, Dr. rer. nat. Dipl.-Chem., DE; Faust, Gottfried, Dr. rer. nat. Dipl.-Chem., DE; Morgenstern, Evelino, Dr. rer. nat. Dipl.-Biol., DE; Scharfenberg, Peter, Dr. rer. nat. Dipl.-Chem., DE
(73) Arzneimittelwerk Dresden GmbH, O - 8122 Radebeul DE

(54) Verfahren zur Herstellung von N-substituierten 3-Aminopyrrolen

(55) Antikonvulsivum; Analgetikum; Arzneimittel; ZNS-Wirksamkeit; 3-Aminopyrrole; Herstellung; Cyclisierung; Trimethiniumsalze; Ausgangsstoffe

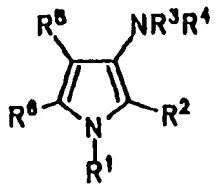
(57) Die Erfindung betrifft ein Verfahren zur Herstellung von N-substituierten 3-Aminopyrrolen der allgemeinen Formel I, die größtenteils neu sind und bisher in dieser Stoffklasse unbekannte pharmakologische Eigenschaften, insbesondere antikonvulsive beziehungsweise analgetische Wirkung, besitzen. Die Erfindung verfolgt das Ziel, größtenteils neue N-substituierte 3-Aminopyrrole, insbesondere solche mit in dieser Stoffklasse bisher unbekannten antikonvulsiven oder analgetischen Eigenschaften zu entwickeln. Erfindungsgemäß werden N-substituierte 3-Aminopyrrole der allgemeinen Formel I durch Cyclisierung von Trimethiniumsalzen erhalten. Die Verbindungen besitzen ausgesprochene antikonvulsive und auch analgetische Wirkung. Formel I



ISSN 0433-6461

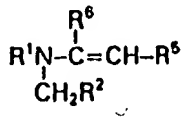
Patentansprüche:

1. Verfahren zur Herstellung von N-substituierten 5-Aminopyrrolen der allgemeinen Formel I,



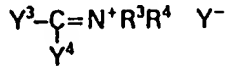
I

in der
R^1 für Wasserstoff, ein unsubstituiertes oder substituiertes Alkyl, ein unsubstituiertes oder substituiertes Cycloalkyl, Aralkyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest, ein Acyl, Alkoxycarbonyl, ein N-un-, N-mono- oder N,N-disubstituiertes Aminocarbonyl oder ein Aminothiocarbonyl,
R^2 für Wasserstoff, Formyl, Acyl, Oxycarbonyl, Alkoxycarbonyl, Aryloxycarbonyl, ein N-un-, N-mono oder N,N-disubstituiertes Aminocarbonyl oder Aminothiocarbonyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest, eine Cyano- oder Nitrogruppe,
R^3 für Wasserstoff, substituiertes oder unsubstituiertes Alkyl, Cycloalkyl, Aralkyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest
R^4 gleich oder verschieden von R^3 für substituiertes oder unsubstituiertes Alkyl, Cycloalkyl, Aralkyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest oder R^3 und R^4 zusammen für eine Alkylbrücke, die auch Sauerstoff, Schwefel oder Stickstoff als Ringatome enthalten kann,
R^5 für einen unsubstituierten oder substituierten Aryl- oder Hetarylrest
oder R^5 und R^6 zusammen für eine Alkylbrücke und R^6 für Wasserstoff, einen Alkyl oder Arylrest oder ein Halogen steht, **dadurch gekennzeichnet**, daß ein Enamin der allgemeinen Formel II,



II

mit der für R^1, R^2, R^5 und R^6 beziehungsweise R^5/R^6 erklärten Bedeutung
mit einem Iminiumsalz der allgemeinen Formel III



III

mit der für R^3 und R^4 beziehungsweise R^3/R^4 erklärten Bedeutung und in der Y^3 und Y^4 gleich oder verschieden Abgangsgruppen, wie beispielsweise Chlor, Aminogruppen, Alkylmercapto-, Trifloxy- oder Alkoxygruppen, und Y^- ein Säurerestanion, beispielsweise ein Halogenid, ein Sulfonat, ein Sulfat oder ein Triflat, darstellen, und gegebenenfalls mit einer Base umgesetzt wird.
2. Verfahren nach Anspruch 1, **dadurch gekennzeichnet**, daß als Base beispielsweise ein Amin, ein Alkali- oder Erdalkalihydroxid oder -hydrid, ein Alkalicarbonat oder ein Metallamid verwendet wird.

Anwendungsgebiet der Erfindung

Die Erfindung betrifft ein Verfahren zur Herstellung von N-substituierten 3-Aminopyrrolen. Die Erfindung ist in der pharmazeutischen und chemischen Industrie sowie in der Humanmedizin einsetzbar.

Charakteristik des bekannten Standes der Technik

Eine antikonvulsive Wirkung an 3-Aminopyrrolen ist bisher nicht bekannt. Es ist beschrieben, daß 3-Aminopyrrole, die in 4-Stellung Aminocarbonyl- (DE 2605419) oder Carbonylgruppen (US 4 198502) tragen als ZNS-wirksame Substanzen eingestuft wurden. Diese Wirkung ist konkret als sedierend und analgetisch benannt aber durch keinerlei Testergebnisse belegt. Die Synthese dieser Verbindungen erfolgte durch Modifizierung von Aminopyrrolderivaten, die ihrerseits aus α-Aminonitrilen und β-Dicarbonylverbindungen gewonnen wurden (DE 2605419, DE 2439284, DE 2462967, DE 2462966, DE 2462963, GB 1492663,

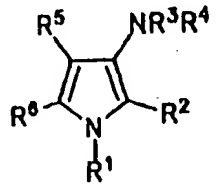
US 4 198502). Sechs Vertreter von 3-Morpholino-4-arylpyrrolcarbonsäureestern mit einem stark eingegrenzten Substituentenmuster sind durch Cyclisierung von 3-Alkoxycarbonylmethylamino-2-arylthioacrylsäuremorpholiden hergestellt worden (A. Knoll, J. Liebscher: Khim. Geterotsikl. Soedin. 1985, 628). Über eine pharmakologische Wirkung derartiger Verbindungen ist bisher nichts bekannt. 3-Amino-4-arylpyrrole, deren Aminogruppe jedoch unsubstituiert ist, wurden durch Reduktion zugehöriger 3-Nitropyrrole gewonnen (J. M. Tedder, B. Webster: J. Chem. Soc. 1960, 3270).
3-Amino-2,4-diphenylpyrrol entsteht bei der Kondensation von Phenacylamin mit sich selbst (S. Gabriel: Ber. dtsch. Chem. Ges. 41 (1908) 1127).
Die bekannten Verfahren beschreiben keine an der Aminogruppe substituierten 3-Amino-4-arylpyrrole, die eine antikonvulsive Wirkung besitzen. Die Substituentenvariabilität der bekannten Verfahren ist stark eingeschränkt.
Die bekannten Antikonvulsiva besitzen den Nachteil von unerwünschten Nebenwirkungen (z.B. Neurotoxizität).

Ziel der Erfindung

Die Erfindung hat das Ziel, ein Verfahren zu entwickeln, daß es gestattet, N-substituierte 3-Aminopyrrole mit in dieser Stoffklasse bisher nicht bekannten pharmakologischen Eigenschaften zugänglich zu machen.

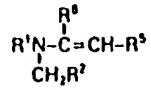
Darlegung des Wesens der Erfindung

Aufgabe der Erfindung ist die Entwicklung eines Verfahrens zur Herstellung von N-substituierten 3-Aminopyrrolen mit bisher in dieser Stoffklasse nicht bekannten pharmakologischen Eigenschaften, insbesondere mit antikonvulsiver oder analgetischer Wirkung. Dabei wird angestrebt, geringere Nebenwirkungen, z.B. eine geringere Neurotoxizität, zu erreichen als bei den derzeitig üblichen Antikonvulsiva.
Erfindungsgemäß, wird diese Aufgabe dadurch gelöst daß N-substituierte 3-Aminopyrrole der allgemeinen Formel I,



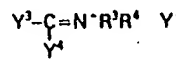
I

in der
R^1 für Wasserstoff, ein unsubstituiertes oder substituiertes Alkyl, ein unsubstituiertes oder substituiertes Cycloalkyl, Aralkyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest, ein Acyl, Alkoxycarbonyl, ein N-un-, N-mono- oder N,N-disubstituiertes Aminocarbonyl oder ein Aminothiocarbonyl,
R^2 für Wasserstoff, Formyl, Acyl, Oxycarbonyl, Alkoxycarbonyl, Aryloxycarbonyl, ein N-un-, N-mono oder N,N-disubstituiertes Aminocarbonyl oder Aminothiocarbonyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest, eine Cyano- oder Nitrogruppe,
R^3 für Wasserstoff, substituiertes oder unsubstituiertes Alkyl, Cycloalkyl, Aralkyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest
R^4 gleich oder verschieden von R^3 für substituiertes oder unsubstituiertes Alkyl, Cycloalkyl, Aralkyl, einen unsubstituierten oder substituierten Aryl- oder Hetarylrest oder R^3 und R^4 zusammen für eine Alkylbrücke, die auch Sauerstoff, Schwefel oder Stickstoff als Ringatome enthalten kann,
R^5 für einen unsubstituierten oder substituierten Aryl- oder Hetarylrest
oder R^5 und R^6 zusammen für eine Alkylbrücke und R^6 für Wasserstoff, einen Alkyl oder Arylrest oder ein Halogen steht,
hergestellt werden durch Umsetzung eines Enamins der allgemeinen Formel II,



II

mit der für R^1, R^2, R^5 und R^6 beziehungsweise R^5/R^6 erklärten Bedeutung
mit einem Iminiumsalz der allgemeinen Formel III



III

mit der für R^3 und R^4 beziehungsweise R^3/R^4 erklärten Bedeutung und in der Y^3 und Y^4 gleich oder verschieden Abgangsgruppen, wie beispielsweise Chlor, Aminogruppen, Alkylmercapto-, Trifloxy- oder Alkoxygruppen, und Y^- ein Säurerestanion, beispielsweise ein Halogenid, ein Sulfonat, ein Sulfat oder ein Triflat, darstellen, und gegebenenfalls mit einer Base, beispielsweise einem Amin, einem Alkali- oder Erdalkalihydroxid oder -hydrid, einem Alkalicarbonat oder einem Metallamid umgesetzt werden.
Die erfindungsgemäßen Verbindungen sind bis auf den 4-(p-Chlorphenyl)-3-morpholinopyrrol-2-carbonsäuremethylester, den 3-Morpholino-4-phenylpyrrol-2-carbonsäuremethylester und -ethylester, den 3-Morpholino-4-(p-tolyl)-pyrrol-2-carbonsäuremethyl und -ethylester sowie den 4-(p-Anisyl)-3-morpholinopyrrol-2-carbonsäuremethylester neu. Die

erfindungsgemäßen Verbindungen zeigen im Test in verschiedenen Krampfmodellen eine hohe antikonvulsive Wirkung, zeichnen sich durch geringe Toxizität und vor allem einen wesentlich höheren protektiven Index aus, als derzeit bekannte handelsübliche Antikonvulsiva. Die antikonvulsive Wirkung ist überraschend, da bisher generell bei 3-Aminopyrrolen keine solche Wirkung beschrieben ist. Die neuen Wirkstoffe können in bekannter Weise in die üblichen Formulierungen überführt werden, wie beispielsweise Tabletten, Kapseln, Dragees, Granulate oder Lösungen unter Verwendung inerter, nicht-toxischer pharmazeutisch geeigneter Trägerstoffe oder Lösungsmittel.
Enamine der allgemeinen Formel II lassen sich in bekannter Weise aus entsprechenden Carbonylverbindungen und Aminen herstellen.
Die Erfindung soll nachstehend an einigen Ausführungsbeispielen erläutert werden.

Ausführungsbeispiele

Beispiel 1

Synthese von N-substituierten 3-Aminopyrrolen der allgemeinen Formel I
Die nach den verschiedenen Varianten hergestellten N-substituierten 3-Aminopyrrole der allgemeinen Formel I sind in Tabelle 1 zusammengestellt.

Variante A
Eine Mischung von 10 mmol Enamin der allgemeinen Formel II, 12 mmol Iminiumsalz der allgemeinen Formel III mit $Y^3 = Y^4 = Y = Cl$ und 20 ml Methylenchlorid wird 2 Stunden unter Rückfluß erhitzt. Dann werden 4 ml Triethylamin zugegeben. Nach nochmaligem 2stündigem Erhitzen unter Rückfluß wird die erkaltete Mischung auf Eis gegossen. Das Endprodukt wird abgesaugt und umkristallisiert.

Variante B
Eine Mischung von 10 mmol Enamin der allgemeinen Formel II, 12 mmol Iminiumsalz der allgemeinen Formel III (Y^3 = Ethylmercapto, Y^4 = Methylmercapto und Y^- = Methusolfat), 15 ml Acetonitril und 4 ml 1,8-Diazabicyclo[5.4.0]-undecen wird 5 Stunden unter Rückfluß erhitzt. Nach dem Abkühlen wird die Lösung auf das halbe Volumen eingeengt und mit wenig Wasser versetzt. Das 3-Aminopyrrol der allgemeinen Formel I wird abgesaugt, mit etwas Wasser gewaschen und umkristallisiert.

Variante C
Eine Lösung von 10 mmol Enamin der allgemeinen Formel II, 10 mmol Iminiumsalz der allgemeinen Formel III mit Y^3 = Morpholino und Y^4 = Cl, und Y^- = Chlorid in 10 ml Acetonitril wird 3 Stunden unter Rückfluß erhitzt. Nach Zugabe einer aus 0,5 g Natrium und 6 ml Ethanol hergestellten Natriumalkoholatlösung wird noch 10 Minuten unter Rückfluß erhitzt. Die abgekühlte Reaktionsmischung wird auf Eis gegossen, und neutralisiert. Das Endprodukt wird abgesaugt und umkristallisiert.

Tabelle 1: Die nach den verschiedenen Varianten hergestellten 3-Aminopyrrole der allgemeinen Formel I

Lfd. Nr.	R^1	R^2	R^3	R^4	R^5	R^6	Schmp. °C	Ausb./ Variante %
I-1	H	CO_2CH_3	CH_3	CH_3	C_6H_5	H	136–137 (Methanol)	38/B
I-2	H	CO_2CH_3	$(CH_2)_2O(CH_2)_2$		C_6H_5	H	179–181 (Methanol)	47/A
I-3	H	CO_2CH_3	$(CH_2)_4$		C_6H_5	H	Öl	45/A
I-4	CH_3	CO_2CH_3	$(CH_2)_2O(CH_2)_2$		C_6H_5	H	86–88 (Methanol)	34/C
I-5	$CH_2CO_2CH_2CH_3$	CO_2CH_3	$(CH_2)_2O(CH_2)_2$		C_6H_5	H	97–98 (Methanol)	32/A
I-6	H	CO_2CH_3	$(CH_2)_2O(CH_2)_2$		4-ClC_6H_4	H	192–193 (Methanol)	41/C
I-7	H	$COCH_3$	$(CH_2)_2O(CH_2)_2$		4-ClC_6H_4	H	172–173 (Methanol)	29/B
I-8	H	$CO_2CH_2CH_3$	H	C_6H_5		$(CH_2)_4$	192–194 (Ethanol)	26/B
I-9	H	CHO	$(CH_2)_2O(CH_2)_2$		4-ClC_6H_4	H	208–210 (Methanol)	29/A
I-10	H	CO_2CH_3	$(CH_2CH_2)_2NCO$-fur-2-yl		4-ClC_6H_4	H	234–236 (Acetonitril)	/A

Beispiel 2

Bestimmung des Schutzes gegen den maximalen Elektrokrampf (MEK)
Durch elektrische Reizung der Vorderpfoten mit einem TUR-Reizstromgerät, Typ RS 12 (Impulsfrequenz 35 Hz, Impulsbreite 20 ms, Tastverhältnis 1:1, Gruppendauer zwischen 400 und 600 ms, Stromstärke der Rechteckimpulse 50 mA) wird bei Mäusen mit einem Gewicht von 18–22 g KM ein Streckkrampf der Hinterextremitäten ausgelöst.
Antikonvulsiva schützen die Tiere vor dem maximalen Elektrokrampf.

Ergebnisse:
Verbindung I-6: bei i.p.-Gabe: $E_{D50} = 3{,}9\ 10^{-5}$ mol/kg
bei p.o.-Gabe: $E_{D50} = 4{,}5 \cdot 10^{-5}$ mol/kg
Verbindung I-3: bei i.p.-Gabe: $5 \cdot 10^{-4}$ mol/kg: 70%
Vergleichswerte: „Carbamazepin": Bei i.p.-Gabe: $L_{D50} = 4{,}3 \cdot 10^{-5}$ mol/kg

Beispiel 3
Bestimmung der Wirkung im pentetrazolinduzierten Krampf
Durch intravenöse Injektion in der Schwanzvene von Mäusen (18–22 g KM) tritt sofort ein Steckkrampf der Hinterextremitäten auf. Die Unterdrückung dieses Krampfbildes gilt als Kriterium für einen antikonvulsiven Effekt der geprüften Substanzen.

Ergebnisse:
Verbindung I-6: bei i.p.-Gabe: $E_{D50} = 4{,}5 \cdot 10^{-6}$ mol/kg
bei p. o.-Gabe: $E_{D50} = 1{,}5 \cdot 10^{-4}$ mol/kg

Beispiel 4
Bestimmung der Krampfschwelle
Durch Infusion von 100mg/kg Pentetrazol
(Infusionsgeschwindigkeit von 36ml/h) über die Schwanzvene treten als erstes klonische Krämpfe (myoclonische Zuckungen) bei Mäusen (18–22 g KM) auf. Die Verlängerung der Infusionsdauer (in s) bis zum Auftreten der Krämpfe im Vergleich zu Kontrolltieren gilt als Erhöhung der Pentetrazolkrampfschwelle und somit als antikonvulsiver Effekt der geprüften Substanzen.

Ergebnisse:
Verbindung I-5: i. p. bei $5 \cdot 10^{-4}$ mol/kg: 20,4% Erhöhung der Krampfschwelle
Verbindung I-4: i. p. bei $5 \cdot 10^{-4}$ mol/kg: 19,4% Erhöhung

Beispiel 5
Bestimmung der orientierenden letalen Dosis
Mäuse (18–22 g KM) erhalten die zu prüfenden Substanzen in Dosierungen von $5 \cdot 10^{-4}$, 10^{-3} und $5 \cdot 10^{-3}$ mol/kg i. V. 24 Stunden post applicationem wird die Letalität der Tiere bestimmt.

Ergebnisse:
Verbindung I-6: oLD größer als $5 \cdot 10^{-3}$ mol/kg

Beispiel 6
Bestimmung der analgetischen Wirkung mit dem Hot Plate Test
Mäuse (18–22 g KM) werden 30 min nach Gabe der Testsubstanzen auf die Heizplatte (hot plate) von 56°C gesetzt, und es wird die Reaktionszeit auf diesen thermischen Schmerzreiz bestimmt. Eine Verlängerung der Reaktionszeit von substanzbehandelten Tieren im Vergleich zu Kontrolltieren wird als analgetischer Effekt gewertet.

Ergebnisse:
Verbindung I-2: p. o. bei 10^{-3} mol/kg: 90% Hemmung (30min p. a.)
Vergleichswert:
Analgin 55% Hemmung

Beispiel 7
Bestimmung der analgetischen Wirkung mit dem Essigsäure-writhing Test
Durch i. p. Gabe von 0,6%iger Essigsäure werden bei Mäusen (18–22 g KM) Bauchdeckenkrämpfe (writhings) ausgelöst. Als Maß für die Wirkstärke einer Substanz dient die Reduktion der Zahl der writhing-Reaktionen behandelter Tiere im Vergleich zur Kontrollgruppe. Neben analgetisch wirksamen Verbindungen senken auch verschiedene ZNS-wirksame Verbindungen die writhings.

Ergebnisse:
Verbindung I-2: p. o. bei 10^{-3} mol/kg 71,3% Hemmung
Vergleichswert:
Analgin: p. o. bei 10^{-4} mol/kg 50% Hemmung

Beispiel 8
Bestimmung der Neurotoxizität mit dem Drehstabmodell
Trainierte Mäuse (18–22 g KM) werden nach Substanzapplikation für 1 min auf den Drehstab (5 Umdrehungen/min) gesetzt. Als Maß für eine Substanzwirkung gilt das vorzeitige Herunterfallen vom Drehstab. Der protektive Index ergibt sich als Quotient von TD_{50}/ED_{50} MEK.

Ergebnis:
Verbindung I-6: $TD_{50} = 1{,}4 \cdot 10^{-3}$ mol/kg; protektiver Index = 36
Vergleichswert:
Carbamazapin: TD_{50} $2{,}2 \cdot 10^{-4}$ mol/kg
Protektiver Index = 5,1

Beispiel 9
Applikationsformen
Für die Applikation werden unter anderem folgende Rezepturen vorgeschlagen:

Kapseln
3-Aminopyrrol der allgemeinen Formel I wird in der erforderlichen Menge in Polyethylenglykol suspendiert und in eine Gelatinemischung der Zusammensetzung

Gelatine	1 Gewichtsteil
Glycerol	5 Gewichtsteile
Wasser	2 Gewichtsteil

eingearbeitet.

Kapseln
Es wird eine Mischung mit folgenden Bestandteilen hergestellt:

Lactose	5 Gewichtsteile
Kartoffelstärke	5 Gewichtsteile
Magnesiumstearat	1 Gewichtsteil

DiesemGemisch wird die entsprechende Menge der Substanz der allgemeinen Formel I zugesetzt.
Die vorgenannten Beispiele sollen die Erfindung näher erläutern, ohne sie einzuschränken. Es sind weitere Zubereitungen als Dragees, Tabletten, Lutschbonbons, Granulat, Pulver, wäßrige Suspension, Sirup und dergleichen möglich.